Pathophysiology of the Liver and Pancreas in Experimentally Produced Acute Pancreatic Necrosis and Therapeutic Effect of Penicillin

AUTHOR(S):

ISHIKAWA, TAKESHI

Pathophysiology of the Liver and Pancreas in Experimentally Produced Acute Pancreatic Necrosis and Therapeutic Effect of Penicillin

by

TAKESHI ISHIKAWA

From the 2nd Department of Surgery, Kanazawa University Medical School
(Director: Prof. Dr. ICHIO HONJO)

Received for Publication Jan. 14, 1965

CONTENT

I. INTRODUCTION

Pathological picture of acute pancreatic necrosis has been generally accepted to be

autodigestion caused by proteolytic enzymes of the pancreas and a sort of intoxication due to proteolytic products, mechanism of which is, however, not yet clearly disclosed. Some investigators[70)74)96)] insist ductal development of pancreatic necrosis caused by regurgitation of activated pancreatic enzymes from the pancreatic duct. It is, however, suspectable to conceive that activation of trypsinogen[56)80)85)] or increase in lipase activity[9)] alone can well explain the occurrence of pancreatic necrosis. REID et al.[79)] did not admit the possibility of occurrence of necrosis by pancreatic proteolytic enzymes such as trypsin. In addition, many assertions have been done to conceive that there precedes circulatory disturbance in glandular tissue[10)37)51)61)75)94)95)]. Concerning the cause of death of this disease, toxication due to destructive products of tissue digestion of trypsin[87)], changes in chemical constitution of blood[1)25)39)52)81)97)109)], decrease in circulating blood volume caused by injuries[80)83)] or increase in permeability of the vessel wall[41)48)51)101)112)] and pancreatic apoplexia[111)] due to thrombus are pointed out. In recent years, WERLE and FORELL[30)106)107)] emphasized collapse of the vessels due to dilatation effect of trypsin on the peripheral vessels. On the other hand, SILER and WULSIN[93)] have insisted that the cause of death cannot be explained merely by anatomical spread of damage in the pancreatic tissue. DRAGSTEDT[21)], LEWIS and WANGENSTEEN[53)] have maintained intoxication due to bacteriotoxin of anaerobic organism, particularly Clostridium welchii which commonly exists in the pancreas, liver and intestine. Furthermore, ULIN and SOKOLIG[101)] also put the greatest emphasis on toxin production of Clostridium welchii which proliferates under hypoxia, ascertaining development of hypovolemia in animals of experimentally produced hemorrhagic pancreatitis.

In parallel with advance in chemotherapy, excellent therapeutic effect of antibiotics for acute pancreatic necrosis and hemorrhagic pancreatitis has been recently recognized[20)42)47)73)77)90)91)]. TSUBOI[99)100)] has suggested that favorable effect of penicillin in acute pancreatitisconsists in inhibition against proteolytic activity of pancreatic enzymes. Except the occasion of bacterial contamination, acute pancreatitis or pancreatic necrosis is generally considered to be non-infectious inflammation, as its principal picture is etiologically nothing but necrosis without bacterial contamination. Therefore, antibiotics are indicated only in the aim of preventing secondary infection. On the other hand, as the studies on mechanism which leads shock syndrome to an irreversible phase make an advancement[15)16)45)54)92)], shock has come to be grasped as an analogical state of general hypoxia following circulatory disturbance, which leads rapidly to characteristic liver hypoxia[60)110)]. It is assumed that irreversible phase of shock is established by vasodepressive material (VDM)[113)] mobilized from the liver or toxin[38)] of anaerobic bacillus emigrated from that organ as its metabolism is impaired. Recent studies[40)50)59)] have revealed that mobilization of VDM and production of bacterial toxin are depressed by the early administration of antibiotics, decrease in hepatic tissue respiration being prevented, and animals can survive.

If the cause of death at acute pancreatitis be largely participated by hypovolemic shock or toxin of anaerobic bacillus proliferating in hypoxic tissue, it is presumed that the metabolic function of the liver is seriously disturbed, particularly, its aerobic metabolism is strongly disturbed from the initial stage, and also that the liver encounters the influence of proteolytic enzymes from the pancreas. At this point, the following experiment was designed to investigate pathophysiology of the liver in the development of experimentally

produced acute pancreatic necrosis, from the aspects of tissue respiration and bacteriology. At the same time, effect of penicillin on the development of the pathological state and the influence of proteolytic activity of trypsin, which was looked on to be a toxic factor, on aerobic metabolism of the liver were studied.

II. MATERIALS AND METHODS

1. Materials and Production of Acute Pancreatic Necrosis

Adult mongrel dogs weighing 7 to 20 kg were used. The abdomen was opened with an upper median incision under intravenous anesthesia of Isozol (15 mg/kg body weight). 1.0 ml/kg body weight of autogenous bile was injected with pressure into the main pancreatic duct of WIRSUNG following the method of ARCHIBALD[5]. The pancreatic duct was then doubly ligated and cut.

2. Measurement of Arterial and Portal Pressures

Arterial pressure was measured with a mercurial manometer connected to the femoral artery, and portal pressure was checked by an aqueous manometer connected with a polyethylene tube inserted to the portal vein through the superior mesenteric vein.

3. Determination of Tissue Respiration of the Liver

Pieces of liver tissue were taken by laparotomy under local anesthesia with 0.5 per cent Xylocaine from the peripheral region[65] of the left inferior lobe before and 8, 16, 24, 48 and 72 hours after the production of acute pancreatic necrosis, respectively. Tissue respiration of these tissue specimens were determined in WARBURG's manometric apparatus by direct method[102)105)]. The piece of liver tissue was immediately immersed in salt phosphate solution by a metthod described by FUJITA[32] and cut into slice not exceeding the thickness of the critical slice with a razor[32]. In the main chamber of the apparatus, 2.5 ml of salt phosphate solution (pH 7.4) containing 0.2 per cent glucose as the respiratory substrate was put. 0.2 ml of NaOH (10 per cent) was given in the center wall, which was covered with rolled filter paper for absorption of CO_2. Air within the respiratory vessel was replaced with oxygen. The temperature was set at 37.5°C, and frequency of shaking at 110 r. p. m. After the determination, the liver slice was dried at 100°C for 24 hours, and the slice was weighed. Oxygen consumption of the tissue was represented in the term of respiratory coefficient (Qo_2).

4. Administration of Penicillin

30×10^4 units of oily procaine penicillin G was administered intramuscularly once a day, 3 times in all, directly after the production of the acute pancratic necrosis, and for succeeding 2 days.

5. Bacteriological Studies of Hepatic and Pancreatic Tissues

a. Anaerobic Culture of Tissue

Some part of tissue was taken from the marginal region of the left inferior lobe of the liver and inferior arm of the pancreas in normal dogs and those of acute pancreatic necrosis. Pieces of the tissue were cut into small fragments aseptically, and cultured in liver-liver bouillon, which was further transfered to blood culture medium of ZEISSLER and plate culture medium of NAGLER modified from WEINBURG's V. F. blood culture medium and cultured for 24 hours under anaerobic condition. From hemolytic reaction, lecithinase

reaction and the colonies of the culture, Clostridium perfringens (welchii) was identified.

b. Determination of Lecithinase C Activity in Tissue and Serum

i. From hepatic and pancreatic tissues taken in a. mentioned above, suspension (5 per cent weight/volume homogenate) in saline solution was prepared, which was centrifuged for 60 minutes at 4000 r. p. m. Amount of α-toxin (lecithinase C) in the supernatant was determined following EVAN's modified method[28] of lecitho-vittelin reaction[58], namely, titer of antitoxin consumed for neutralization of lecithinase activity contained in 1.0 ml of the supernatant (Fig. 1). It is said that 1 unit of antitoxin (Lb) neutralizes 57 mouse LD_{50} of toxin[44]. Lecithin solution was prepared following the method of VAN HEYNINGEN[103]. Obtained value of α-toxin contained in 1.0 g of the tissue was converted to the term of mouse LD_{50}.

ii. Blood was taken from the femoral vein after the production of acute pancreatic necrosis with the lapse of time and α-toxin (lecithinase C) in 1.0 ml of serum was similarly determined.

6. Determination of Proteolytic Activity of Trypsin on Casein and Inhibitory Effect of Penicillin

a. Preparation of Trypsin Solution

Crystalline trypsin 'Trypure Novo' (Novo Industry, Denmark) was used. Crystalline trypsin was solved in salt phosphate buffer solution[32] (pH 7.4) immediately before the experiment.

b. Determination of Proteolytic Activity of Trypsin on Casein

Trypsin activity for casein decomposition was determined following the method of FULD-GROSS[31]. 0.1 per cent casein solution was prepared using Hammasten purified casein (E. Merck Co.). Casein digestion for 60 minutes at 38°C was pursued and the immediately anteceding number of the test tube of cloudy turbidity was adopted as trypsin unit $\left(\mathrm{Tr}\frac{38°}{60'}\right)$. 100, 250, 500, 1,000, 5,000 and 10,000 units of aqueous procaine penicillin was added respectively in each experiment.

No. of Test tube	Control	I	II	III	IV	V	VI	VII	VIII	IX	X	Control
Material (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cl. antitoxin (ml)		(0.1U) 0.1	0.2	0.4	0.8	0.2	0.6					
						(1.0U) 0.1	0.1	0.2	0.4	0.6	0.8	(30U) 0.1
Sailne solution (ml)	0.8	0.7	0.6	0.4		0.5	0.1	0.6	0.4	0.2		0.7
37°C 30min. Water bath												
Solution of Yolk (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
37°C 60min. Water bath												
Lb		0.05	0.1	0.2	0.4	0.6	0.8	1.0	2.0	3.0	4.0	

Fig. 1 Lecithovittelin Test (Modified Method of Evans)

7. Determination of Tissue Respiration under the Influence of Trypsin

Trypsin solution of various concentrations was previously put in the side chamber of the apparatus, and after stirring, tissue suspension was made to contain trypsin in 0.05, 0.25 and 0.5 per cent, respectively. Thus, tissue respiration of the hepatic slice was determined with direct method. Wet weight of the slice was determined, 1/5 of which was considered to be dried weight.

8. Determination of Lecithinase Activity in Pancreatic Juice, Suspension of Pancreatic Tissue, Ethanol Extract of Tissue and Pancreatin

Pancreatic fistula was produced in dogs by inserting polyethylene tube into the main pancreatic duct. Pancreatic juice was taken in the fasting state and 30 minutes after intravenous injection of 1 mg pilocarpine hydrochloride. Five per cent tissue suspension, as in 5. b., 1 per cent tissue extract in saline solution (extracted with 60% ethanol) and 1 per cent pancreatin for control were prepared. Lecithinase activity of each 1.0 ml material was determined by yolk lecithin decomposition, i. e. lecithin decomposition for 60 minutes at 38°C was pursued and immediately anteceding number of the test tube of cloudy turbidity was taken as lecithinase unit $\left(\mathrm{Lc}\frac{38°}{60'}\right)$.

III. RESULTS

1. Changes in Arterial and Portal Pressures and Laparotomy Findings in Dogs of Acute Pancreatic Necrosis

Pancreatic necrosis was produced in 5 animals. Arterial pressure ranged from 100 to 122 mmHg, being 115 mmHg on the average, and portal pressure ranged from 88 to 104 mmH_2O, with the average of 96 mmH_2O, before the production of pancreatic necrosis. After the production of pancreatic necrosis, arterial pressure fluctuated from 5 to 20 mmHg irregularly, with similarly slight fluctuation of 5 to 20 mmH_2O in portal pressure. These flucuations in the pressure restored around the level which was observed before the production of pancreatic necrosis, approximately 30 minutes later, without significant fluctuation thereafter. A tendency of gradual fall in arterial pressure was observed towards 5th hour in a single case, and 6th hour in other 4 cases, after the production of pancreatic necrosis. At the same time, a tendency of elevation of portal pressure was observed (Tab. 1 and 1'). Eight hours after the production of pancreatic necrosis, arterial pressure was 25 mmHg lower, on the average, and portal pressure 40 mmH_2O higher, similarly on the average, than the level before the operation. These changes in arterial and portal pressuree are shown in Fig. 2.

Laparotomy finding revealed an accumulation of large amount of bloody ascites in all cases 6 hours after the production of pancreatic necrosis, with remarkable edema and hemorrhage in the pancreatic tissue and, furthermore, fat necrosis in the surrounding tissues. Such picture developed, gradually aggravating to final necrosis of the pancreatic tissue, leading all 5 animals to death 50 to 70 hours after the production of pancreatic necrosis.

2. Tissue Respiration of the Liver in Dogs of Acute Pancreatic Necrosis

i. Animals without Penicillin Administration

Hepatic Qo_2 until 24 th hour was determined in 3 dogs after the production of pan-

Tab. 1 Changes in Arterial Blood Pressure in Dogs of Acute Pancreatic Necrosis (mmHg)

No. of Dog	Sex	Body weight (kg)	Control	10 min.	20 min.	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.
1	♂	13.0	120	130	128	122	120	120	122	120	104	100	102	96
2	♂	8.0	112	132	108	102	116	120	112	110	108	100	96	90
3	♂	10.0	122	136	130	118	122	124	120	122	120	108	102	98
4	♀	11.0	100	122	112	98	100	102	100	104	98	90	90	74
5	♂	9.5	110	124	100	108	110	112	108	108	110	100	92	80

Tab. 1' Changes in Portal Blood Pressure in Dogs of Acute Pancreatic Necrosis (mmH_2O)

No. of Dog	Sex	Control	10 min.	20 min.	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	Shrvival time (hrs.)
1	♂	104	100	108	100	102	100	98	104	118	124	132	142	54
2	♂	96	90	102	92	96	100	92	98	100	112	122	138	70
3	♂	88	84	90	90	82	86	88	90	88	100	130	132	75
4	♀	90	84	92	92	88	92	90	94	94	108	116	130	72
5	♂	102	92	104	100	96	90	94	100	104	116	128	142	55

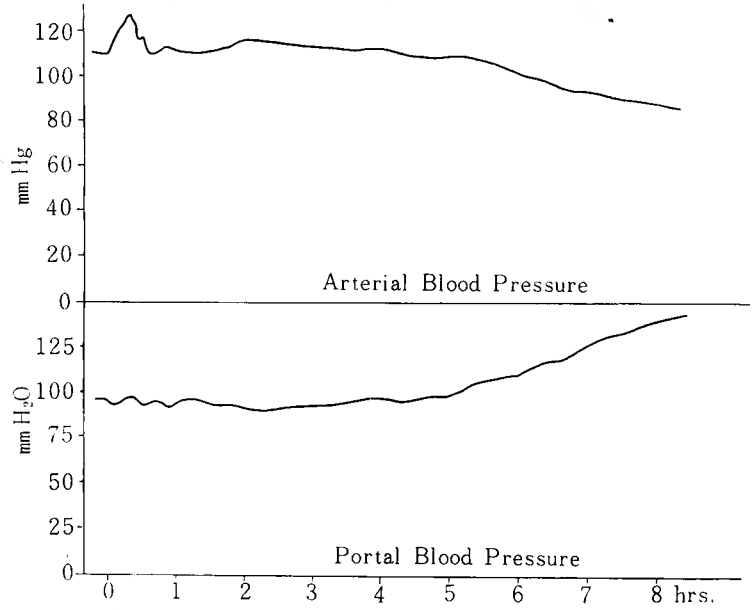

Fig. 2 Changes in Arterial and Portal Pressure in Acute Pancreatic Necrosis. (Mean value of 5 Cases)

creatic necrosis. Before the onset of the disease, it was 9.4 to 10.3, 9.8 on the average, which increased by 4 per cent of normal level to be 9.9 to 10.7, 10.4 on the average, 8 hours after the production of pancreatic necrosis. At 16 th hour, it returned to almost normal level. At 24th hour, it became 8.0 to 8.4, 8.2 on the average, showing decrease of 15 per cent of normal level (Tab. 2). Hepatic Qo_2 of more than 24 hours after the production of pancreatic necrosis was determined in 11 dogs. Before the production of

pancreatic necrosis, it was 8.8 to 10.9, 9.8 on the average, which was followed by gradual decrease as 7.1 to 10.6, 8.3 on the average, at 24th hour, 5.1 to 8.8, 7.7 on the average at 48 th hour, and 4.4 to 9.2, 6.4 on the average at 72nd hour. Hepatic Qo_2 showed gradual decrease with the lapse of time, extent of the decrease being 15 per cent at 24th hour, 22 per cent at 48th hour and 35 per cent at 72nd hour, respectively. Nine animals out of 11 died 72 hours after the onset of the disease, survival rate being as low as 10 per cent (Tab. 3, Fig. 3).

ii. Animals with Penicillin Administration

Hepatic Qo_2 until 24 th hour was determined in 3 dogs after the production of pancreatic necrosis. It was 8.8 to 10.1, 9.7 on the average, before the production of pancreatic necrosis, which was followed by an average increase of 6 per cent of normal level to be 9.8 to 11.2, 10.3 on the average, 8 hours after the production of pancreatic necrosis. At 16 th hour, it decreased slightly to be 9.1 to 9.8, 9.3 on the average. It further decreased by 8 per cent of normal level at 24 th hour to be 8.5 to 9.5, 9.0 on the average (Tab. 4). Hepatic Qo_2 of more than 24 hours after the production of pancreatic necrosis was determined in 11 dogs. It was 9.0 to 10.6, 9.7 on the average, before the production of pancreatic necrosis, which was followed by an decrease as 7.6 to 9.5, 8.7 on the average at 24 th hour, 6.2 to 9.9, 8.1 on the average at 48 th hour and 5.9 to 9.1, 7.8 on the

Tab. 2 Hepatic Qo_2 in Dogs of Acute Pancreatic Necrosis without Administration of Penicillin

No. of Dog	Sex	Body weight (kg)	Before Pancreatic Necrosis	After 8 hrs.	16 hrs.	24 hrs.
6	♂	8.0	9.4	9.9	9.4	8.1
7	♂	9.5	9.7	10.6	10.0	8.4
9	♀	9.5	10.3	10.7	9.8	8.0
Mean value			9.8	10.4	9.7	8.2
Rate of Qo_2 change				+4%		−15%

Tab. 3 Hepatic Qo_2 in Dogs of Acute Pancreatic Necrosis without Administration of Penicillin

No. of Dog	Sex	Body weight (kg)	Before Pancreatic Necrosis	After 24 hrs.	48 hrs.	72 hrs.	Outcome
20	♂	8.0	9.9	8.5	7.7	†	died
21	♀	9.0	10.1	10.6	8.8	9.2	survived
22	♀	10.0	9.8	7.9	5.5	†	died
23	♀	10.0	9.6	8.2	6.2	4.8	died
24	♂	12.0	10.5	9.0	7.8	5.6	died
25	♀	7.5	10.9	8.3	6.2	4.4	died
26	♀	7.5	8.8	7.8	†		died
27	♀	8.0	9.7	7.1	5.1	†	died
28	♂	10.0	9.3	7.8	7.2	6.4	died
29	♀	10.5	9.6	8.0	7.0	6.2	died
30	♂	12.5	10.1	8.8	7.8	8.8	survived
Mean value			9.8	8.3	7.7	6.4	
Rate of Qo_2 decrease				15%	22%	35%	

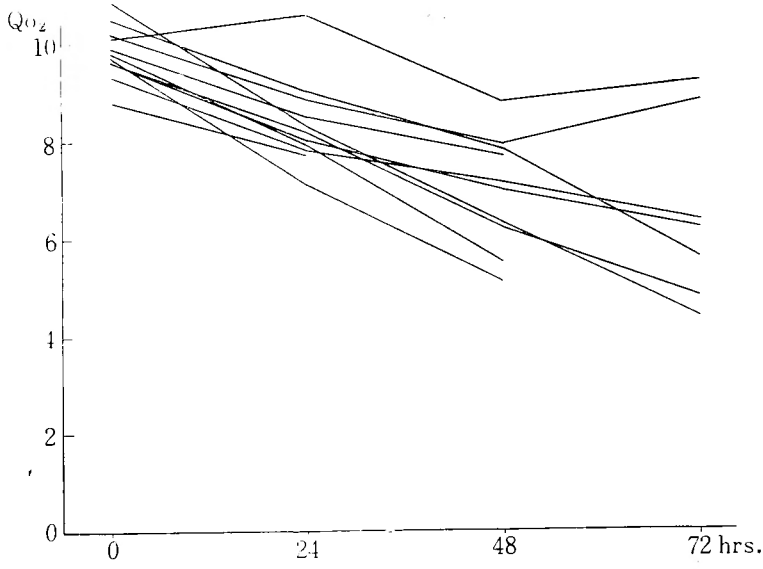

Fig. 3 Changes in Tissue Respiration of the Liver in Acute Pancreatic Necrosis without Treatment

Tab. 4 Hepatic Qo_2 in Dogs of Acute Pancreatic Necrosis with Administration of Penicillin

No. of Dog	Sex	Body weight (kg)	Before Pancreatic Necrosis	After 8 hrs.	16 hrs.	24 hrs.
10	♂	10.5	9.2	9.8	9.1	8.5
11	♀	9.0	8.8	10.1	9.2	9.0
13	♂	9.5	10.1	11.2	9.8	9.5
Mean value			9.7	10.3	9.3	9.0
Rate of Qo_2 change				+6%	−4%	−8%

average at 72nd hour. Extent of decrease in hepatic Qo_2 was 10 per cent at 24th hour, 17 per cent at 48th hour and 21 per cent at 72nd hour, which was invariably more slight than the decrease in animals without administration of penicillin. Until 72nd hour of the disease, death occured in 4 cases, survival rate being as increased as to 64 per cent (Tab. 5, Fig. 4).

3. Bacteriological Studies of Hepatic and Pancreatic Tissue

a. Anaerobic Culture of Tissue

i. Normal Animals

Hepatic and pancreatic tissues of 5 normal dogs were cultured. In all cases, hemolytic reaction and lecithinase reaction could be recognized. Numerous colonies of Clostridium perfringens (welchii) were observed. On the other hand, these tissues were taken from 3 dogs, and cultured 24 hours after intramuscular administration of 30×10^4 units of penicillin. Hemolytic reaction and lecithinase reaction were positive, and growth of Clostridium perfringens could be observed also (Tab. 6 and 7).

ii. Animals of Acute Pancreatic Necrosis without Penicillin Administration

Tab. 5 Hepatic Qo_2 in Dogs of Acute Pancreatic Necrosis with Administration of Penicillin

No. of Dog	Sex	Body weight (kg)	Before Pancreatic Necrosis	After 24 hrs.	48 hrs.	72 hrs.	Outcome
31	♂	9.0	9.6	9.2	8.5	8.0	survived
32	♂	14.0	9.0	8.2	6.2	†	died
33	♂	7.5	10.6	9.4	8.0	8.5	survived
34	♀	12.0	9.5	8.8	9.0	8.4	survived
35	♀	10.5	10.2	9.1	8.5	7.8	shrvived
36	♂	9.0	10.2	9.5	9.9	8.2	survived
38	♂	7.5	9.8	8.8	7.9	7.0	died
39	♀	7.5	9.4	7.6	6.6	†	died
40	♂	12.0	10.0	8.8	8.2	7.5	survived
42	♀	11.0	9.8	8.5	7.9	5.9	died
43	♀	8.0	9.0	7.8	8.8	9.1	survived
Mean value			9.7	8.7	8.1	7.8	
Rate of Qo_2 decrease				10%	17%	21%	

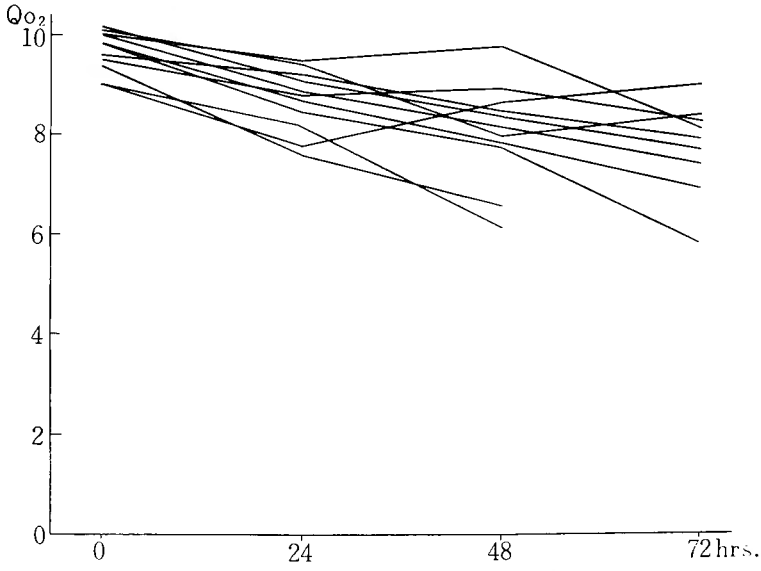

Fig. 4 Changes in Tissue Respiration of the Liver in Acute Pancreatic Necrosis with Penicillin Treatment

Pancreatic necrosis was produced in 3 dogs and hepatic and pancreatic tissues were taken and provided for anaerobic culture 6, 12, 18, 24 and 48 hours after the production of pancreatic necrosis. Hemolytic reaction and lecithinase reaction were invariably positive in every material and Clostridium welchii proliferated also in every material (Tab. 8 and 9).

iii. Animals of Acute Pancreatic Necrosis with Penicillin Administration

Studies were carried out in 3 dogs. Hemolytic reaction, lecithinase reaction and growth of Clostridium welchii could be observed in all cases (Tab. 8 and 9).

Tab. 6 Anaerobic Culture of Liver Tissue of Dogs

No. of Dog	Sex	Body weight (kg)	Dosis of Penicillin (Unit)	Zeissler's Culture Medium		Nagler's Modified Cultured Medium	
				Hemolysis	Cl. welchii	Lipolysis	Cl. welchii
75	♂	15.0	—	(+)	(+)	(+)	(+)
76	♀	15.0	—	(+)	(+)	(+)	(+)
77	♂	15.5	—	(+)	(+)	(+)	(+)
79	♂	11.5	—	(+)	(+)	(+)	(+)
80	♂	12.5	—	(+)	(+)	(+)	(+)
82	♀	10.0	30×10^4	(+)	(+)	(+)	(+)
83	♀	10.5	30×10^4	(+)	(+)	(+)	(+)
84	♀	11.5	30×10^4	(+)	(+)	(+)	(+)

Tab. 7 Anaerobic Culture of Pancreas Tissue of Dogs

No. of Dog	Sex	Body weight (kg)	Dosis of Penicillin (Unit)	Zeissler's Culture Medium		Nagler's Modified Cultured Medium	
				Hemolysis	Cl. welchii	Lipolysis	Cl. welchii
85	♂	17.0	—	(+)	(+)	(+)	(+)
86	♀	12.5	—	(+)	(+)	(+)	(+)
87	♂	8.0	—	(+)	(+)	(+)	(+)
88	♂	16.0	—	(+)	(+)	(+)	(+)
90	♀	11.0	—	(+)	(+)	(+)	(+)
91	♀	15.5	30×10^4	(+)	(+)	(+)	(+)
92	♂	16.5	30×10^4	(+)	(+)	(+)	(+)
93	♀	11.5	30×10^4	(+)	(+)	(+)	(+)

Tab. 8 Anaerobic Culture of Liver Tissue in Acute Pancreatic Necrosis

No. of Dog	Sex	Administration of Penicillin	Culture of Clostridium welchii				
			6 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.
75	♂	—	(+)	(+)	(+)	(+)	(+)
76	♀	—	(+)	(+)	(+)	(+)	(+)
77	♂	—	(+)	(+)	(+)	(+)	(+)
79	♂	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)
80	♂	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)
81	♀	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)

Tab. 9 Anaerobic Culture of Pancreas Tissue in Acute Pancreatic Necrosis

No. of Dog	Sex	Administration of Penicillin	Culture of Clostridium Welchii				
			6 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.
85	♂	—	(+)	(+)	(+)	(+)	(+)
86	♀	—	(+)	(+)	(+)	(+)	(+)
87	♂	—	(+)	(+)	(+)	(+)	(+)
88	♂	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)
89	♀	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)
90	♀	30×10^4U. for 3 days	(+)	(+)	(+)	(+)	(+)

b. Lecithinase Activity of Tissue and Serum

i. Hepatic and Pancreatic Tissues in Normal Animals

In all 5 dogs, lecithinase activity could not be observed in the liver tissue, while intense activity of lecithinase could be observed invariably in the pancreatic tissue (Tab. 10).

Tab. 10 Lecitho-vittelin Test of Normal Liver and Pancreas Tissues

No. of Dog	Sex	Body weight (kg)	Lecithovittelin Reaction	
			Liver tissue	Pancreas tissue
94	♂	10.5	(−)	(++)
95	♀	9.5	(−)	(++)
96	♂	8.5	(−)	(++)
98	♂	11.0	(−)	(++)
99	♀	12.0	(−)	(++)

ii. Animals of Acute Pancreatic Necrosis without Penicillin Administration

In 8 dogs pancreatic necrosis was produced and activity of lecithinase C originating from Clostridium welchii was determined with the lapse of time. Six hours after the onset of the disease, lecithinase activity could not be detected in the liver in any case. At 12th hour of the disease, activity of lecithinase C could be demonstrated in the liver of 4 cases out of 8, it being 112 mouse LD_{50} in 2 cases and 224 mouse LD_{50} in 2 other cases. At 18th hour of the disease, activity of lecithinase C could be demonstrated in the liver of all 8 cases. With the only exception of dog No. 95, activity of lecithinase C increased as time went on and death occurred in 7 cases out of these 8 (Tab. 11).

Lecithinase C activity was also determined in peripheral blood of experimental animals with the lapse of time. The activity, however, could not be found in any case and at any time.

iii. Animals of Acute Pancreatic Necrosis with Penicillin Administration

Pancreatic necrosis was produced in 7 dogs and lecithinase C activity was determined with the lapse of time. At 6th hour of the disease, lecithinase C activity could not be demonstrated in the liver of all cases. The activity was observed in the liver of 3 cases out of 7, 12 hours after the onset of the disease, it being 112 mouse LD_{50} in 2 cases and 224 mouse LD_{50} in one. At 18th hour of the disease, lecithinase C activity could be observed in the liver of 6 cases, it being found at 24th hour in the remaining 1 case. Five animals survived in which the activity decreased or disappeared as time went on,

Tab. 11 Intrahepatic Lecithinase C Activity in Dogs of Acute Pancreatic Necrosis without Treatment

No. of Dog	Sex	Body weight (kg)	Amount of Produced Lecithinase C (mouse LD_{50}/g)					Survivals (hrs.)
			6 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.	
75	♂	15.0	—	224	896	1,120	1,120	55
76	♀	15.0	—	—	224	896	1,120	70
77	♂	15.0	—	112	224	896	1,120	68
78	♂	11.5	—	224	448	1,120	1,120	57
86	♀	12.5	—	—	224	672	1,120	54
95	♀	10.5	—	—	112	448	112	survived
97	♂	9.5	—	112	224	896	2,240	48
98	♀	10.0	—	—	112	224	896	75

whereas 2 animals, in which the activity increased gradually, died (Tab. 12). Similarly to the control animals, lecithinase C activity could not be demonstrated in peripheral blood of all the experimental animals at any time.

Tab. 12 Intrahepatic Lecithinase C Activity in Dogs of Acute Pancreatic Necrosis treated with Penicillin (30×10^4Units for 3 days)

No. of Dog	Sex	Body weight kg	Amount of Produced Lecithinase C (mouse LD_{50}/g) 6 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.	Outcome
82	♀	10.0	—	112	224	224	112	survived
83	♀	10.5	—	224	672	896	1,120	died
84	♀	11.5	—	—	112	224	—	survived
88	♂	16.0	—	—	—	224	—	survived
90	♀	11.0	—	112	224	224	—	survived
91	♀	15.5	—	—	224	—	—	survived
92	♂	16.5	—	—	112	448	1,120	died

4. Proteolytic Activity of Trypsin on Casein and Inhibitory Effect of Penicillin

Proteolytic activity of 10.0mg of crystalline trypsin on casein i. e. trypsin unit $\left(\mathrm{Tr}\frac{38°}{60'}\right)$ was 2^6. In casein solution containing aqueous penicillin (100, 250, 500, 1,000, 5,000, 10,000 units, respectively), $\mathrm{Tr}\frac{38°}{60'}$ was 2^6, 2^5, 2^4, 2^5, 2^6 and 2^5, suggesting that proteolytic activity of trypsin was scarcely inhibited by penicillin (Tab. 13).

Tab. 13 Proteolytic Activity of Crystalline Trypsin (10.0 mg) and Inhibitory Effect of Penicillin (Fuld-Gross' Method)

0.1% Casein solution pH : 7.4 (ml)	Penicillin aqueous. (Unit)	$\mathrm{Tr}\frac{38°}{60'}$
2.0	—	2^6
2.0	100	2^6
2.0	250	2^5
2.0	500	2^4
2.0	1,000	2^5
2.0	5,000	2^6
2.0	10,000	2^5

5. Inhibitory Effect of Trypsin on Tissue Respiration

Hepatic Qo_2 in 0.05 per cent trypsin solution showed decrease of 4 per cent or increase of 5 per cent compared with Qo_2 in control, showing an increase of 1.5 per cent on the average. In the solution of 0.25 per cent, it showed increase of 3 to 9 per cent, 5.4 per cent on the average. It increased by 3 to 11 per cent in the solution of 0.5 per cent, 6.2 per cent increase on the average. However, these changes in oxygen consumption cannot be accepted to be significant compared with hepatic Qo_2 in normal animals, revealing at least no inhibitory effect on tissue respiration (Tab. 14).

Tab. 14 Influence of Trypsin on Tissue Oxygen Consumption (Qo_2) of the Liver in Normal Dogs. Substrate : 0.2% Glucose, pH : 7.4

No. of Dog	Hepatic Qo_2	0.05% Trypsin	0.25% Trypsin	0.5% Trypsin
51	9.4	+4%	+6%	+ 3%
52	9.2	−1	+4	+ 5
54	10.2	—	+5	+11
55	10.2	+5	+9	+ 5
57	8.9	−2	+3	+ 7

6. Lecithinase Activity of Pancreatic Juice, Suspension of Pancreatic Tissue, Ethanol Extract of Pancreatic Tissue and Pancreatin

Lecithinase activity was determined in the material from 5 dogs and crude pancreatin, and an average Lc$\frac{38^\circ}{60'}$ of the pancreatic juice in the fasting state was 2^4. The activity of pancreatic juice after the injection of 1mg pilocarpine markedly increased to Lc$\frac{38^\circ}{60'}$ of 2^{10}. The activity of 5 per cent suspension of pancreatic tissue was 2^9, on the average, and in 1 per cent tissue extract in saline solution Lc$\frac{38^\circ}{60'}$ was 2^7, on the average. Finally, in 1 per cent solution of pancreatin Lc$\frac{38^\circ}{60'}$ was 2^4, on the average (Tab. 15).

IV. DISCUSSION

Characteristic feature of acute pancreatic necrosis, a serious type of acute pancreatitis, is wide-spread necrosis of the pancreatic tissue and shock state accompanied by exudation of corporeal fluid. It has long been pointed out that besides its proteolytic activity, trypsin has pharmacodynamic effect[30)72)84)] to lower arterial pressure, effect of alterating coagulation mechanism[24)29)52)97)] and chemical constitution[25)] of blood and effect of increasing permeability or injuring of the vassel wall[80)83)].

In the present experiment, dogs of acute pancreatic necrosis produced following the method of ARCHIBALD[5)] showed the tendency of gradual fall of arterial pressure and elevation of portal pressure from 6 hours after the onset of the disease, which was also accompanied by the accumulation of bloody ascites. These changes developed in parallel with the course of the disease and finally necrosis occurred in the pancreas.

Tissue respiration is the most basic metabolism of organism to maintain its life and it is oxygen consumption in the reaction performed by oxidation-reduction enzymes within the cells. Tissue respiration comes to decay generally in the state of general anoxia or circulatory collapse. WILHELMI[108)] observed in rats that hepatic tissue respiration decreased in proportion to the length of time of the interruption of the hepatic proper artery. RUSSELL[86)] also noticed that hepatic tissue respiration decreased depending upon the degree of shock, in the experiment of hemorrhagic shock in rat. In the present experiment, aerobic

Tab. 15 Lecithinase Activity $\left(\text{Lc}\frac{38^\circ}{60'}\right)$ in Pancreatic Juice, Pancreatin, Surpension of Pancreatic Tissue and 60% Ethanol Extract of the Pancreas (each 1.0 ml)

No.	Pancreatic Juice in Fasting	Pancreatic Juice after the Injection of 1.0 mg Pilocarpine	1 % Pancreatin	5 % Suspension of Pancreas	1 % Extract of Pancreas
1	2^4	2^{11}	2^4	2^8	2^7
2	2^4	2^{10}	2^3	2^8	2^6
3	2^3	2^{10}	2^4	2^9	2^6
4	2^4	2^9	2^4	2^9	2^5
5	2^4	2^{10}	2^5	2^9	2^7
Mean	2^4	2^{10}	2^4	2^9	2^7

metabolism (tissue respiration) increased slightly 8 hours after the production of pancreatic necrosis, which, however, decreased in parallel with the development of the disease thereafter. In other words, if trypsin drains into the liver through portal flow[19)43)76)], the liver comes to be exposed continuously to circulatory disturbance caused by trypsin pharmacodynamically[84)], finally being led to serious anoxia, with resulting disturbance of various function of liver cell, particularly disturbance of aerobic metabolism. Accordingly, the degree of aerobic metabolism can be deemed as indicative of the severity of the disease. From another standpoint, it is suggested that the state of decreased hepatic tissue respiration is less resistant against hypoxia. On the other hand, it is accepted that hepatic anoxia enhances the mobilization of VDM (anaerobic reductive ferritin) or bacterial toxin which establishes irreversible phase of shock[16)60)92)114)]. In general, occurrence of liver failure at acute pancreatic necrosis obviously presumed from retention of B. S. P.,[12)] hyperlipemia[13)34)] or hypoprothrombinemia[13)]. MIRSKY and FREIS[62)] reported that repeatedly performed intravenous administration of trypsin resulted in shock, including hepatorenal failure, the liver being congested and swollen with scattered focal necrosis.

In the present experiment, mortality in animals without administration of penicillin was 90 per cent, which was lessened to 36 per cent by penicillin administered immediately after the onset of the disease, decrease in hepatic tissne respiration also being held in only a slight degree. This finding is obviously accepted to demonstrate more slight disturbance of aerobic metabolism of the liver in cases with administration of penicillin. Jndging from the above mentioned effect of penicillin, bacterial toxin, particnlarly that of anaerobic bacillus, Clostridium welchii is pointed out as a cause of death in pancreatic necrosis[14)21)27)28)83)89)]. ULIN and SOKOLIG[101)] did not seek the cause of death in pancreatic necrosis in specific toxicity of proteolytic enzymes of the pancreas itself nor in the non-specific intoxication due to proteolytic product, but they ascertained development of hypovolemia and their attention was attracted to anoxia or the toxin of Clostridium welchii which proliferated as the result of anoxia. In this respect, KEITH[48)] reported a decrease of 33 per cent in circulating blood volume and that of 30 per cent in the red blood cell in the patients of acute pancreatitis. Since α-toxin (lecithinase C) of Clostridinm welchii, which is said to be lethal toxin, increases permeability[57)] of capillaries and cell membrane and inactivates respiratory enzymes of the cells[26)], enhancing necrosis or hemolysis[28)], it plays an important role in the development of irreversible shock[15)45)54)]. Here, the curative effect of penicillin is understood to be attributable to the antibiotic or bactericidal inhibition of toxin production[90)91)]. However, LEWIS and WANGENSTEEN[53)] observed unexpectedly higher mortality in acute pancreatitis produced after commonly existing bacteria was sterilized, and they found that the effect of pretreatment with polyvalent antitoxin was inferior to that of penicillin administered towards the onset of the disease. There are some investigators[6)98)] who do not attribute the mechanism of penicillin effect in anoxia solely to the bactericidal effect.

Common existence of Clostridium welchii was ascertained in 100 per cent by anaerobic culture of hepatic and pancreatic tissue of dog. Clostridium welchii could be invariably found in the culture of the normal tissues 24 hours after the administration of penicillin. Results of the culture were also invariably positive after the production of acute pancreatic necrosis, regardless of administration of penicillin, α-toxin (lecithinase C) being demon-

strated in the liver 12 to 24 hours later. The activity of lecithinase C was, however, lower in animals of penicillin administration than those of control. In addition, lecithinase C disappeared earlier in experimental animals and these animals survived. Clostridium welchii forms spore under aerobic condition and proliferates and produces toxin as the condition changes to anaerobic one due to hypoxia. Among various toxin produced by Clostridium welchii, α-toxin was identified to be lecithinase C by VAN HEYNINGEN[103], which was produced at proliferative phase. Production of the toxin is influenced by various conditions such as pH of the culture medium or Fe concentration. PAPPENHEIMER[71] reported that the lower the concentration of non-hemin Fe, the more toxin production was enhanced. MIHASHI[2)63] also observed descension of pH and of oxidation reduction potentiality and proliferation of bacteria, 2 hours after the interruption of blood flow to the muscles. NAGLER[64] observed the maximum toxin production at 5th hour of the culture of Clostridium welchii, and maintained that the liver was mostly suited to the growth of Clostridium welchii.

It is understandable that penicillin inhibited the growth of Clostridium welchii in the present experiment. However, it is presumed from invariable existence of Clostridium welchii demonstrated regardless of administration of penicillin, that penicillin did not necessarily lessen activity of lecithinase C in the liver simply by its bactericidal effect, but the growth was replaced by formation of spore due to the change brought about by penicillin in the physical and chemical conditions suited to the growth of anaerobic bacillus. It is frequently observed in cases of gas gangrene that the growth of Clostridium welchii is explosive, however, it ceases its growth rapidly as the environment is improved[4]. On the other hand, it is said that bactericidal effect of penicillin acts at the logarithmic phase of growth[4]. NAKASE[65] demonstrated that mobilization of ferritin of the liver could be prevented after the interruption of the hepatic artery by penicillin administered immediately after it. KUBOTA[50] also observed that penicillin could prevent decrease in aerobic metabolism by estimating tissue respiration in favorite site of liver necrosis following the interruption of the hepatic artery. As these effects of penicillin can be observed already in the stadium in which toxin of Clostridium welchii, which proliferates in the anoxic liver after the interruption of the hepatic artery[40)110], has nothing to do with the disease process, it is presumed that the effect of penicillin rather consists in the inhibition of pathogenic potential, as postulated by ALTEMEIER[4], than bactericidal activity.

Cells of the tissue perform respiration under well arranged connection of oxidation and reduction enzymes. However, cell respiration is inhibited, when the close relationship of the enzymes is disturbed, even if certain enzymes are not affected. As a cause of gradual decrease in tissue respiration of the liver at acute pancreatic necrosis in the present experiment, proteolytic activity of trypsin against cellular construction can be pointed out, besides hypoxia in the liver caused by a pharmacodynamic fall of blood pressure due to trypsin liberated from the pancreas. BATTELLI and STERN[8] observed that trypsin extracted from the pancreas had inhibitory effect on oxidation process in the tissue, and suggested destruction of apo- or holoferment of succinoxidase group. On the other hand, it is admitted that trypsin has extremely weak activity against natural protein and viable cells[3], and DRAGSTEDT[22] observed insusceptibility of normal viable cells to this enzyme. In the

present experiment, hepatic tissue respiration was not affected in vitro by the coexistence of trypsin. REID et al[79]. also insisted that production of oxidative energy was remarkably inhibited in homogenate of the liver, kidney and heart by pancreas homogenate or pancreatic vagal juice, but the inhibitory effect could hardly be observed by the use of crystalline trypsin or crystalline chymotrypsin and they maintained that the organized tissues such as in the form of slice were not affected by trypsin. In other words, viable cells are insusceptible to trypsin and it is presumed that in the process of pancreatic necrosis or decrease in hepatic tissue respiration these changes are brought about by an decrease in blood supply for the maintenance of metabolism of the tissue cells due to fall of blood pressure or hypovolemia as a result of vasospasm which are pharmacodynamically caused by intrapancreatic activation or liberation should be emphasized rather than enzymochemical effect.

Although TSUBOI[99)100)] suggested that clinical effect of penicillin consists in the inhibition of trypsin activity or lipase, CRONE-MUNZEBROCK[17)] asserted that inhibitory effect for pancreatic enzymes as trypsin or lipase could not be demonstrated and effect of penicillin seen in pancreatic necrosis would be attributed to the bactericidal effect. In the present experiment also, proteolytic activity of crystalline trypsin in casein digestion was hardly affected by addition of penicillin of various dosis.

From the finding of invariable existence of Clostridium welchii in the liver and pancreas and that of decrease in mortality of dogs in pancreatic necrosis caused by administration of penicillin, bacterial toxin lecithinase C (α-toxin) originating from anaerobic bacillus might be considered to be a factor of lethality in pathophysiology of the liver and pancreas at acute pancreatic necrosis. In the present experiment, intense lipolytic activity on lecithin was demonstrated in the pancreatic tissue or pancreatic juice. Lecithinase C[26)] is produced by anaerobic clostridium proliferating in anaerobic condition of the tissue and different from pancreatogenic lecithinase (phospholipase) in its origin. In 1932, BELFANTI and NIKUNI[67)] smilarly discovered in the pancreas an enzyme which decomposed lecithin. SCHMIDT[88)] identified this enzyme in the pancreas to be lecithinase (phospholipase) A and B. Furthermore, NYGAARD and SUMMER[69)] observed that pancreatogenic lecithinase, i. e. lecithinase A, as well as lecithinase C of Clostridium welchii, entirely inactivated oxidation activity of succinate oxidase or cytochrom oxidase, and ascertained that abundantly contained lecithin as cement substance[7)] between these two enzymes was destructed in this process. Existence of lecithinase in the pancreatic tissue and pancreatic juice enables to presume strong possibility that this enzyme is closely related as well as lecithinase C, to a series of pathophysiology such as development of pancreatic necrosis and hemorrhage or hypovolemic shock following liberation of the enzyme. Pancreatogenic lecithinase is also contained in various snake toxin as crotoxin[58)], but it is probable that lecithinase A closely associates with necrotic process or hypovolemic shock similarly to lecithinase C, since lecithinase A originally exists in the pancreatic tissue and juice. Based on the fact that the oxidation of succinate oxidase is strongly inhibited by pancreatic vagal juice, REID et al.[79)] had a concept concerning the mechanism in the development of pancreatic necrosis that it was attributable to the fact that the reaction of protein molecule synthesizing enzymes (inducer) in glandular cells, which normally synthesize pancreatic excretory enzymes (digestive enzymes), proceeds in the opposite direction of the synthesis. But there is no study on other

enzymes than proteolytic ones. Hence, BLUMENTHAL and PROBSTEIN[11] are sceptic of this concept, because existence of inactivating substances of respiratory enzyme (succinoxidase) also in vagal juice and at development of wide-spread necrosis in the intraperitoneal adipose tissue besides the pancreas at pancreatic necrosis cannot be explained by inverse reaction of digestive enzyme inducers.

However, in the present experiment, lecithinase activity could not be demonstrated by lecithovittelin reaction in circulating blood at any stadium of pancreatic necrosis. FURR et al.[33] reported that detection of lecithinase C in blood stream by the use of lecithovittelin reaction was unsuccessful even after intravenous injection of the enzyme of large amount exceeding lethal dosis.

GRONCHI[35] observed that lecithinase A is ethanol soluble and DRAGSTEDT[23)104] extracted pancreatic antifatty liver hormone (lipocaic) with 60 per cent ethanol. REID et al.,[79] as mentioned in the above, observed existence of respiration inhibiting substance in vagal juice. All these findings are assumed to be closely related to the observation of the present experiment that lecithinase (phospholipase) could be demonstrated in the pancreatic juice and pancreatic homogenate, and a high activity of lecithinase (A) was observed in 60 per cent ethanol extract and in the pancreatic juice after the injection of pilocarpine.

Physiological significance and excretion process of pancreatic lecithinase are not clarified. It may be excreted through the duct as digestive enzyme. However, judging from the fact that lecithinase activity cannot be detected except proliferating phase of Clostridium welchii in the normal liver, the most important organ in the intermediate metabolism of lipids, it is presumed that besides its effect as exocrine digestive enzyme pancreatic lecithinase may possibly participate in the intermediate process of the metabolism of compound lipids according to the principle of 'exocrine-endocrine partition'[46)82]. It is reported that fatty liver develops in totally depancreatized dogs with insulin treatment[23)104], and it is also said that in essential hyperlipemia[49] observed in relapsing pancreatitis, and atheromatous arteriosclerosis[78], disturbance of pancreatic function can be observed as well as abnormality and dysfunction of intermediate metabolism of lipids. On the other hand, it is reported that pancreatitis can be produced by feeding animals with high fat and low protein diet[36)55]. Accordingly, the facts such as increased permeability due to pancreatic lecithinase, hemorrhage associated to the destruction of phospholipids[18] in cellular membrane and inactivation[66] of thrombokinase, inactivation of dehydrogenase and electrone transfer system in respiratory enzymes should also be pointed out in the pathological process of pancreatic necrosis.

V. SUMMARY

Pathophysiology or the cause of death in acute pancreatic necrosis is not well clarified, and the pancreas itself remains to be an interesting and attractive organ to many physiologists. As a factor in cause of death or in the development of pancreatic necrosis, damage of the tissue caused by autodigestion of pancreatic digestive enzymes, particularly that of trypsin and hypovolemic shock have been most emphasized.

In the present experiment, acute pancreatic necrosis was produced and influence of penicillin on hepatic tissue respiration was studied. The results obtained are summarized

in the below.

1. After the production of acute pancreatic necrosis, blood pressure fell gradually and portal pressure elevated progressively from 6 th hour of the disease. At this stadium, hemorrhage and edema in the pancreatic tissue and necrosis in the surrounding adipose tissue were observed. Bloody exudate accumulated in the peritoneal cavity. These findings aggravated gradually.

2. Approximately 72 hours after the production of pancreatic necrosis, 90 per cent of experimental animals died, whereas the mortality was as low as 36 per cent when penicillin was administered from the moment immediately after the onset of the disease.

3. Tissue respiration of the liver in acute pancreatic necrosis decreased progressively from 24th hour of the disease. On the contrary, the decrease in tissue respiration of the liver was in a slight degree in animals with penicillin administration.

4. Clostridium welchii that produces lethal α-toxin (lecithinase C) could be commonly demonstrated in the liver and pancreas of dogs regardless of the administration of penicillin.

5. In the development of acute pancreatic necrosis, it was 12 to 18 hours after the onset of the disease that lecithinase C was produced in the liver, which gradually increased on. On the other hand, in animals of penicillin administration also, lecithinase C was demonstrated in the liver 12 to 18 hours after the onset of the disease. However, the activity was relatively low and it disappeared shortly.

6. Lecithinase activity was not demonstrated in normal liver tissue whereas in the pancreatic tissue remarkable activity of lecithinase could be observed.

7. Proteolytic activity of trypsin was not affected by penicillin.

8. Tissue respiration of the liver was not affected by trypsin in vitro.

9. In the ethanol extract from the pancreatic juice and pancreatic tissue, lecithinase activity was observed, and the activity in the pancreatic juice markedly increased after the injection of pilocarpine.

As summarized in the above, after the production of acute pancreatic necrosis, gradual decrease in tissue respiration of the liver and increase in lecithinase C activity due to growth of Clostridium welchii in the liver were observed. By the administration of penicillin, decrease in tissue respiration in the liver was lessened and increase in lecithinase C activity was also inhibited, with resulting decrease in mortality of experimental animals. On the other hand, it is presumed that existence of pancreatic lecithinase A in the pancreatic juice and pancreatic tissue having the similar toxicity as the bacterial lecithinase C has an important significance as well as trypsin in the development of panceratic necrosis and hypovolemia.

In accomplishing the present paper, the author is greatly indebted to Prof. Dr. Ichio Honjo for his enthusiastic and kind guidances and encouragements throughout the course of the experiment, the author is also grateful to Prof. Dr. Shoki Nishida in the Dept. of Bacteriology in the University for his valuable advices for bacteriological studies.

VI. REFERENCES

1) Abrams, W. W.: Spontaneous intravascular clotting in acute pancreatitis. J. Kan. Med. Soc., **55**: 379, 1954.
*2) Akabori, S. (Edit): Seikagaku-Kooza 11. Kyoritsu-shuppan, Tokyo, 1959.

*3) Akabori, S. (Edit) : Koosokenkyuho II. III. Asakura-shoten, Tokyo, 1956.
4) Altemeier, W. A., Furste, W. L. and Culbertson, W. R. : Chemotherapy in gas gangrene. An experimental study. Arch. Surg., **55** : 668, 1947.
5) Archibald, E. W. : The experimental production of pancreatitis in animals as the result of the resistance of the common duct sphincter. Surg. Gynec. & Obst., **28** : 529, 1919.
6) Baez, S., Srikantia, S. G., Fobes, I. and Shorr, E. : Metabolic action of aureomycin on liver ; Inhibition of anaerobic release of ferritin. Fed. Proc., **14** : 6, 1955.
7) Ball, E. G. and Cooper, O. : The activity of succinate oxidase in relation to phosphate and phosphorus compounds. J. Biol. Chem., **180** : 113, 1949.
8) Battelli, F. and Stern, L. : Wirkung des Trypsins auf die verschiedenen Oxydationsvorgänge in den Tiergeweben. Biochem. Z., **34** : 263, 1911.
9) Baxter, H., Baxter, S. G. and McIntosch, J. F. : Variation in levels of serum lipase in experimental pancreatitis. Am. J. Digest. Dis., **5** : 423, 1938.
10) Block, M. A., Wakim, K. G. and Baggenstoss, A. H. : Experimental studies concerning factors in the pathogenesis of acute pancreatitis. Surg. Gynec. & Obst., **99** : 83, 1954.
11) Blumenthal, H. T. and Probstein, J. G. : Pancreatitis. Charles C. Thomas Publisher, Springfield, Illinois 1959.
12) Bockus, H. L., Kalser, M. H., Roth, J. L. A., Bogoch, A. L. and Stein, G. : Clinical features of acute inflammation of the pancreas. A. M. A. Arch. Int. Med., **96** : 308, 1955.
13) Bockus, H. L. and Raffensperger, E. C. : Acute pancreatitis. New York J. Med., **48** : 2252, 1948.
14) Bratt, H. : Gasbacilleninfektion des Pancreas und Pankreasnekrose. Virch. Arch. f. Path. Anat., **246** : 33, 1923.
15) Broitman, S. A., Bezman, A. L., Hazel, M. M. and Zamcheck, H. : Effect of endotoxin on gastrointestinal mucosa of rat. Proc. Soc. Exp. Biol. Med., **100** : 557, 1959.
16) Chambers, R., Zweifach, B. W., Lowenstein, B. E. and Lee, R. E. : Vaso-excitor and -depressor substances as "toxic" factors in experimentally induced shock. Proc. Soc. Exp. Biol. Med., **56** : 127, 1944.
17) Crone-Münzebrock. : Experimentelle Untersuchungen zur Pankreasfermentinactivierung mit Penicillin. Klin. Wschr., **29** : 550, 1951.
18) Dawson, R. M. C. : The animal phospholipase, their structure, metabolism and biological significance. Biol. Rev., **32** : 188, 1957.
19) Doerr, W. : Fermententglesung im Pankreas, pathologischanatomisch Gesehen. Ärztl. Wochschr., 681, 1953.
20) Donhauser, J. L. : Acute pancreatitis. A review of 86 cases. Am. J. Physiol. **88** : 946, 1954.
21) Dragstedt, L. A., Haymond, H. E. and Ellis, J. C. : Pathogenesis of acute pancreatitis (acute pancreatic necrosis). Arch. Surg., **28** : 232, 1934.
22) Dragstedt, L. R. : The digestion of living tissues by proteolytic anzymes. Science., **98** : 131, 1943.
23) Dragstedt, L. R., van Prohaska, J. and Harms, H. P. : Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. Am. J. Physiol., **117** : 175, 1936.
24) Eagle, H. and Harris, S. T. : Studies in blood coagulation. I. The coagulation by proteolytic enzyme. J. Gen. Physiol., **20** : 543, 1937.
25) Edmondson, H. A. and Berne, C. J. : Calcium changes in acute pancreatic necrosis. Surg. Gynec. & Obst., **79** : 240, 1944.
26) Edwards, S. W. and Ball, E. G. : The action of phospholipases on succinate oxidase and cytochrome oxidase. J. Biol. Chem., **209** : 619, 1954.
27) Ellis, J. C. and Dragstedt, L. R. : Liver autolysis in vivo. A. M. A. Arch. Surg., **20** : 8, 1930.
28) Evans, D. G. : The in-vitro production of -toxin, -hemolysin and hyaluronidase by strains of Cl. welchii type A, and the relationship of in-vitro properties to virulence for guinea-pigs. J. Path. Bact., **57** : 75, 1945.
29) Ferguson, J. H. and Erickson, B. N. : The coagulant action of crystalline trypsin. Proc. Roy. Soc. London, **125** : 204, 1938.
30) Forell, M. M. : Zur Frage des Entstehungsmechanismus der Kreislaufkollapses bei der akuten Pankreasnekrose. Gastroenterologia., **34** : 225, 1955.
*31) Fujii, Y. : Methods of Biochemistry ; Determination Nanzando Co. Tokyo., 1946.
*32) Fujita, S. : Manometric technique and its applications in medical and biological studies. Tokyo Iwanamisyoten, 2nd Edt. 1949.

33) Furr, W. E., Bourdeau, R. V., Roach, H. D. and Laufman, H.: In vivo effects of clostridium welchii lecithinase. Surg. Gynec. & Obst., **95** : 465, 1953.
34) Gardner, C. E. and Fawcett, B.: Acute pancreatitis with hyperlipemia. Surgery, **27** : 512, 1950.
35) Gronchi, V.: Ricerche sulla lecitinasi "A" del pancreas. Sperimentale, **90** : 223, 1936.
36) Grossman, M. I., Greengard, H. and Ivy, A. C.: The effect of dietary composition on pancreatic enzymes. Am. J. Physiol., **138** : 676, 1943.
37) Guleke, N.: Über die experimentelle Pankreasnekrose und die Todesursache bei akuten Pankreaserkrankungen. Arch. f. klin. Chir., **85** : 615, 1908.
38) Hershey, S. G., Zweifach, B. W. and Antopol, W.: Factors associated with protection against experimental shock. Anesthesiology, **17** : 265, 1956.
39) Hiruma, K.: Studien über die Beziehung einzelner Organe zur Blutgerinnung und über die Beeinflussung der Thrombinwirkung durch Sekrete und Excrete, nebst Untersuchungen über Antithrombin und über Fibrinolyse. Biochem. Ztschr., **139** : 152, 1923.
*40) Honjo, I.: Pathophysiology of the hepatic artery. J. Jusenigakukai, **63** : 333, 1959.
41) Howard, J. M.: Surgical physiology of pancreatitis. Surg. Clin. of North America, **29** : 1789, 1949.
42) Howard, J. M., James, C. L. and Evans, S. S.: The excretion of sulfonamide and antibiotics in pancreatic juice. Surg. Forumof 1951. p. 581, W. B. Saunders Co., Phila.
43) Howard, J. M., Smyth, A. K. and Pekes, J. J.: Acute pancreatitis. Pathways of enzymes into the blood stream. Surgery, **26** : 161, 1949.
*44) Ishida, S., Yamagishi, T., Kawai, Y. and Nishida, S.: On toxinogenicity of clostridia. I. Isolation of toxinogenic Cl. welchii. Medicine and Biology, **62** : 41, 1962.
45) Jacob, S., Weizel, H., Gordon, E., Korman, H., Schweinburg, F. B., Frank, H. A. and Fine, J.: Bacterial action in development of irreversibility to transfusion in hemorrhagic shock in dogs. Am. J. Physiol., **179** : 523, 1954.
46) Janowitz, H. D. and Hollander, F.: The exocrine-endocrine partition of enzymes in the digestive tract. Gastroenterol, **17** : 591, 1951.
47) Jones, C. A.: Medical management of pancreatitis. A. M. A. Arch. Int. Med., **96** : 332, 1956.
48) Keith, L. M. and Watman, R. N.: Blood volume deficits in pancreatitis. Surg. Forum., **380** : 5, 1954.
49) Klatskin, G. and Gordon, M.: Relationship between relapsing pancreatitis and essential hyperlipemia. Am. J. Med., **12** : 3, 1952.
50) Kubota, H.: Experimental studies on the respiration of the liver tissue after the interruption of the hepatic artery in dogs. Arch. Jap. Chir., **30** : 661, 1961.
51) Langenbecks.: Experimenteller Beitrag zur Wirkung des Trypsin auf die Gefasse. Arch. f. klin. Chir., **93** : 279, 1910.
52) Lasher, E. P. and McCabe, M. M.: Changes in coagulation time with experimental acute pancreatitis in dogs. Arch. Surg., **60** : 164, 1950.
53) Lewis, F. J. and Wangensteen, O. H.: Antibiotics in the treatment of experimental acute hemorrhagic pancreatitis in dogs. Proc. Soc. Exp. Biol. Med., **74** : 453, 1950.
54) Lilleher, R. C.: Intestinal factor in irreversible endotoxin shock. Ann. Surg., **148** : 513, 1958.
55) Lindsay, S., Entenman, C. and Chaikoff, I. L.: Pancreatitis accompanying hepatic disease in dogs a high fat, low protein diet. Arch. Path., **45** : 635, 1948.
56) Löwenthal, K.: Pathogenese der akuten Pankreasnekrose. Deutsche Med. Wschr., **58** : 1209, 1932.
57) Macfarlene, M. G.: The biochemistry of bacterial toxins. 2. The enzymatic specificity of Clostridium welchii lecithinase. Biochem., J. **42** : 588, 1948.
58) Macfarlene, R. G., Oakley, C. L. and Anderson, C. G.: Haemolysis and the production of opalescence in serum and lecithovittelin by the α-toxin of Clostridium welchii. J. Path. Bact., **52** : 99, 1941.
59) Markowitz, J., Rappaport, A. and Scott, A. C.: Prevention of liver necrosis following ligation of hepatic artery. Proc. Soc. Exp. Biol. and Med., **70** : 305, 1949.
60) Mazur, A. and Shorr, E.: Hepatorenal factors in circulatory homeostasis. IX. The identification of the hepatic vasodepressor substance, VDM. with ferritin. J. Biol. Chem., **176** : 771, 1948.
61) Meuguy, R. B., Hallenbeck, G. A., Bollman, J. L. and Grindlay, J. H.: Ductal and vascular factors in etiology of experimentally induced acute pancreatitis. Arch. Surg., **74** : 881, 1957.
62) Mirsky, I. A. and Preis, E. D.: Renal and hepatic injury in "Trypsin shock". Proc. Soc. Exp' Biol. Med. **57** : 278, 1944.

*63) Mihashi, S.: Pathogenic Bacteriology, Nanzando Co. Tokyo, 1961.
64) Nagler, E. P. O.: Observations on a reaction between the lethal toxins of Cl. welchii (type A) and human serum. Brit. J. Exper. Path., **20** : 473, 1939.
65) Nakase, A.: On the cause of the liver necrosis after interruption of hepatic artery in dogs. Arch. Jap. Chir., **29** : 157, 1960.
66) Neumann, W. und Habermann, E.: Beitrage zur Charakterisierung der Wirkstoffe des Bienengiftes. Arch. expl. Path. u. Pharmakol., **222** : 367, 1954.
67) Nikuni, Z.: On the formation of lysolecithin from eggyolk lecithin by pancreas extract. Bull. Agr. Chem. Soc. Japan, **8** : 104, 1932.
68) Nygaard, A. P. and Summer, J. B.: The effect of lecithinase A on the succinoxidase system. J. Biol. Chem., **200** : 723, 1953.
69) Oakley, C. L.: Gas gangrene Brit. Med. Bull., **10** : 52, 1954.
70) Opie, E. L.: The relation of cholelithiasis to disease of the pancreas and to fat necrosis. Am. J. Physiol., **121** : 27, 1901.
71) Pappenheimer, A. M. and Shaskan, E.: Effect of iron on carbohydrates metabolism of Clostridium welchii. J. Biol. Chem., **155** : 265, 1944.
72) Peck, M. E.: Preliminary observations on the effect of trypsin administered intravenously. J. A. M. A., **154** : 15, 1954.
73) Persky, L., Schweinburg, F. B., Jacob, S. and Fine, J.: Aureomycin in experimental acute pancreatitis in dogs. Surgery, **30** : 652, 1951.
74) Popper, H. L.: Pankreassaft in dem Gallenwegen. Seine pathogenetische Bedeutung für die Entstehung der akuten Pankreaserkrankungen. Arch. f. klin. Chir., **175** : 660, 1933.
75) Popper, H. L., Necheles, H. and Russell, K. C.: Transition of pancreatic edema into pancreatic necrosis. Surg. Gynec. & Obst., **87** : 78, 1948.
76) Popper, H. L. and Necheles, H.: Pathways of enzymes into the blood in acute damage of the pancreas. Proc. Soc. Exp. Biol. Med., **43** : 220, 1940.
77) Pristley, J. T., Taylor, L. M. and Rogers, J. D.: Surgical treatment of chronic relapsing pancreatitis. Surgery, **37** : 317, 1955.
78) Probstein, J. G., Joshi, R. A. and Blumenthal, H. T.: Atheromatous embolization. An etiology of acute pancreatitis. A. M. A. Arch. Surg., **75** : 566, 1957.
79) Reid, L. C., Paulette, R. E., Challis, T. W. and Hinton, J. W.: The mechanism of the pathogensis of pancreatic necrosis and the therapeutic effect of propyothiouracil. Surgery, **43** : 538, 1958.
80) Rich, A. R. and Duff, G. L.: Experimental and pathological studies on the pathogenesis of acute hemorrhagic pancreatitus. Bull. Johns Hopkins Hosp., **58** : 212, 1936.
81) Richman, A.: Acute pancreatitis Am. J. Med., **21** : 216, 1956.
82) Richterich, R.: Physiologie der Pankreas-Sekretion. Bibl. Gastroentero., **1** : 107, 1961.
83) Roberts, N. J., Baggenstoss, A. H. and Confort, M. W.: Acute pancreatic necrosis. A clinicopathologic study. Am. J. Clin. Path., **20** : 742, 1950.
84) Rocha e Silva, M.: Beitrage zur Pharmakologie des Trypsins. II. Wirkung des Trypsins auf den Blutkreislauf bei Katze, Kaninchen und Hund. Arch. f. Exper. Path. u. Pharm., **194** : 351, 1940.
85) Rush, B. and Clifton, E. F.: The role of trypsin in the pathogenesis of acute hemorrhagic pancreatitis, and the effect of antitrypsin agent in treatment. Surgery, **3** : 349, 1952.
86) Russell, J. A., Long, C. N. H. and Wilhelmi, A. E.: Biochemical studies on shock. IV. The oxygen consumption of liver and kidney tissue from rats in hemorrhagic shock. J. Exper. Med., **79** : 23, 1944.
87) Schmieden, V. and Sebening, W.: Surgery of the pancreas, with especial consideration of acute pancreatic necrosis. Surg. Gynec. & Obst., **46** : 735, 1928.
88) Schmidt, G., Hershman, B. and Thannhauser, S. J.: The isolation of α-gryceerylphosphorylcholine from incubated beef pancreas: Its significance for the intermediary metabolism of lecithin. J. Biol. Chem., **161** : 523, 1945.
89) Schmidt, F. O, Bacr, R. S. and Ponder, E. J.: Optical properties of red cell membrane. J. Cell Comp. Physiol., **9** : 89, 1936.
90) Schweinburg, F. B., Jacob, S., Persky, L. and Fine, J.: Further studies on the role of bacteria in death from acute hemorrhagic pancreatitis in dogs. Surgery, **33** : 367, 1953.
91) Schweinburg, F. B., Yashar, Y., Aphrahamian, H. A., Davidoff, D. and Fine, J.: Resistance to bacteria in hemorrhgic shock, decline in phagocytosis promoting capacity of serum in shock. Proc. Soc. Exp. Biol.

Med., 88 : 587, 1955.
92) Shorr, E., Zweifach, B. W., Furchgott, R. F. and Baez, S. : Hepatorenal factor in circulatory homeostasis ; tissue origins of vasotropic principles VEM and VDM, which appear during evolution of hemorrhagic and tourniquet shock. Circulation., **3** : 42, 1951.
93) Siler, V. E. and Wulsin, J. H. : Consideration of the lethal factors in acute pancreatits. Arch. Surg., **63** : 146, 1951.
94) Smyth, C. J. : Etiology of acute hemorrhagic pancreatitis with special reference to vascular factors. Arch. Path., **30** : 651, 1940.
95) Stein, A. A., Powers, S. R. and Browne, H. H. : Experimental hemorrhagic pancreatitis new concepts of pathogenesis. Ann. Surg., **143** : 508, 1956.
96) Stering, J. A. : The common channel for bile and pancreatic duct. Surg. Gynec. & Obst., **98** : 420, 1954.
97) Tagnon, H. J. : The nature of the mechanism of the shock produced by the injection of trypsin and thrombin. J. Clin. Invest., **24** : 1, 1945.
98) Thomas, L. : Physiological disturbances produced by endotoxins. Ann. Rev. Physiol., **16** : 467, 1954.
*99) Tsuboi, A. : Therapeutic effect of penicillin in chemotherapy in acute pancreatic necrosis (acute pancreatitis). Geka, **12** : 168, 1950.
*100) Tsuboi, A. : Influence of penicillin and various sulfas on digestive activity of human pancreatic enzymes in acute pancreatic necrosis (acute pancreatitis). Geka, **12** : 374, 1950.
101) Ulin, A. W. and Sokolig, I. H. : Mortality factors in experimental hemorrhagic pancreaittis. Gastroenterology, **38** : 781, 1960.
102) Umbreit, W. W., Burris, R. H. and Stauffer, J. F. : Manometric techniques and tissue metabolism. Burgess Publishing Co., Minneapolis, 1951.
103) van Heyningen, W. E. : The biochemistry of the gas gangrene toxins. I. Isolation of the -toxin of Clostridium welchii. Biochem. J., **35** : 1246, 1941.
104) van Prohaska, J., Dragstedt, L. R. and Harms, H. P. : The relation of pancreatic juice to the fatty infiltration and degeneration of the liver in the depancreatized dog. Am. J. Physiol, **117** : 166, 1936.
105) Warburg, O., Posener, K. und Negelein, E. : Über den Stoffwechsel der Carzinomzelle. Biol., Z. **152** : 309, 1924.
106) Werle, E., Forell, M. M und Mauer, L. : Zum Mechanismus der Blutdruckwirkung des Trypsins. Naturwissenschaften, **24** : 627, 1953.
107) Werle, E., Forell, M. M und Mauer, L. : Zur Kenntnis der blutdrucksenden Wirkung des Trypsins. Arch. exp. Path. Pharmakol, **225** : 369, 1955.
108) Wilhelmi, A. E., Russell, J. A., Engel, F. L. and Long, C. N. : The effects of hepatic anoxia on the respiration of liver slices in vitro. Am. J. Physiol, **144** : 669, 1945.
109) Williams, G. : Acute pancreatic necrosis as a cause of sudden death. Brit. M. J., **1** : 1184, 1954.
110) Yamabe, I. : Study on lecithinase C activity in the liver necrosis after interruption of the arterial flow to the liver. Arch. Jap. Chir., **29** : 205, 1960.
111) Zenker, F. A. : Über tödliche Pankreasblutung. Dtsch. Zschr. f. prakt. Med., **1** : 351, 1874.
112) Zollinger, R. M., Keith, L. M and Ellison, E. H. : Pancreatitis. New Engl. J. Med., **251** : 497, 1954.
113) Zweifach, B. W., Hershey, S. G., Guccione, I., Sephra, I and Antopol, W. : Bacterial involvement in reaction to stress in rat. Fed. Proc., **15** : 206, 1956.
114) Zweifach, B. W., Benacerraf, B. and Thomas, L. : Relationship between vascular manifestations of shock produced by endotoxin, trauma and hemorrhage ; possible role of reticulo-endothelial system in resistance to each type of shock. J. Exp. Med., **106** : 403, 1957.

(*in Japanese)

和文抄録

実験的急性膵壊死の肝、膵病態生理と penicillin の効果

金沢大学医学部外科第2講座（主任：本庄一夫教授）

石　川　　威

急性膵壊死の病態生理の特徴は広汎な膵組織の壊死と体液の滲出を伴う shock 症状である．これらの病態の発現機序は必らずしも明らかにされたわけでなく，近来，trypsin の蛋白分解作用のほかに，末梢血管を虚脱する薬力学的作用が注目されている．また，Dragstedt, Lewis & Wangensteen, Ulin & Sokolig らは，hypovolemic shock ないし hypoxia の結果，嫌気性桿菌（Cl. welchii）が増殖するとして，死因には菌毒素（α-toxin）を注目している．即ち，Siler & Wulsin は膵自体の解剖学的病像より，全身的障害を強調している．

Hypovolemic shock が発現すれば肝の代謝機能ことに好気的代謝に重大な影響が現われることは，一般 shock の研究で明らかにされた．

急性出血性膵炎や急性膵壊死でも肝機能が傷害され，他方，抗生物質の投与が膵壊死の死亡率を減少させてきている．そこで，実験的急性膵壊死において，逸脱する膵酵素や，低酸素症の影響を最も強く受ける肝の病態生理や膵組織の壊死発現の機序を組織呼吸並びに細菌学的見地から追求し，併せて病勢に対する penicillin の効果を検討し，次の結果を得た．

1）急性膵壊死を作成すると，概ね6時間後より血圧は徐々に下降し，遂に門脈圧は進行性に亢進する傾向が認められた．作成後6時間の膵組織には浮腫および出血と周囲の脂肪組織に壊死が認められた．腹腔内には血性滲出液が貯溜し，これらの症状は漸次増強した．

2）急性膵壊死作成後，ほぼ72時間で実験犬は90%死亡した．これに反して，作成直後から penicillin を投与した場合，死亡率は36%に減少した．

3）急性膵壊死犬の肝組織呼吸は，作成後24時間頃から進行性に低下した．これに反して，penicillin 投与例は肝組織呼吸の低下が軽度であつた．

4）犬の肝および膵内には致死的毒素といわれるα毒素（lecithinase C）を産生する Welch 菌が常在し，penicillin を投与しても菌培養成績では常に陽性であつた．

5）急性膵壊死過程において，肝内に lecithinase C が産生されるのは，作成後12～18時間頃で，以後，菌毒素量は漸次増高した．一方，penicillin 投与例でも肝内菌毒素は，膵壊死作成後12～18時間に検出されたが，毒素活性は低く，比較的早期に消失する傾向を示した．

6）正常肝組織では，lecithinase 活性が検出されたかつたが，膵組織では常に lecithinase 活性は著しかつた．

7）trypsin の casein 分解活性は penicillin で阻害されなかつた．

8）肝組織呼吸は，in vitro で trypsin によつて阻害されなかつた．

9）膵液および膵組織の ethanol 抽出物には lecithinase 活性が認められた．さらに，pilocarpine 注射後の膵液では，活性が著明に増加した．

以上，実験的に急性膵壊死を作成すると，肝組織呼吸は漸次低下して肝内に Welch 菌のα毒素（lecithinase C）が増加したが，penicillin を投与することにより肝組織呼吸の低下が軽減された．その結果，肝内 lecithinase C 活性の増加も抑制されて，実験犬の死亡率は低下した．一方，膵液および膵組織内に Welch 菌毒素と同様に作用する lecithinase（phospholipase）Aが存在することから，膵壊死発現や hypovolemic shock の病態に lecithinase A が trypsin と共に重要な役割を演ずるものと推測される．